EpiQuik™ In Situ Histone H3-K9 Acetylation Assay Kit

Base Catalog # P-4004

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik™ In Situ* Histone H3-K9 Acetylation Assay Kit is suitable for specifically measuring histone H3-K9 acetylation *in situ* using cultured adherent cells.

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KIT CONTENTS

Components	96 assays P-4004-096
GH1 (10X Wash Buffer)	30 ml
GH2 (Permeabilizing Buffer)	30 ml
GH3 (Blocking Buffer)	20 ml
GH4 (Antibody Buffer)	15 ml
GH5 (Capture Antibody, 1 mg/ml)*	6 µl
GH6 (Detection Antibody, 400 µg/ml)*	20 µl
GH7 (Developing Solution)	12 ml
GH8 (Stop Solution)	6 ml
30% H_2O_2 Solution	0.5 ml
Acetylated H3-K9 Control (20 µg/ml)*	15 µl
8-Well Control Strips	2
Microplates	1

** For maximum recovery of the products, centrifuge the original vial prior to opening the cap.*

SHIPPING & STORAGE

The kit is shipped in two parts: one part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **GH6** and **Acetylated H3-K9 Control** at –20°C; (2) Store **GH8** at room temperature away from light; (3) Store **all other components** at 4°C away from light. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: Check if buffers, **GH1** and **GH4**, contain salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffers until the salts are re-dissolved.

GENERAL PRODUCT INFORMATION

Usage Limitation: The *EpiQuik™ In Situ* Histone H3-K9 Acetylation Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

Quality Control: Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design.

Intellectual Property: *EpiQuik™* is a trademark of Epigentek Group Inc.

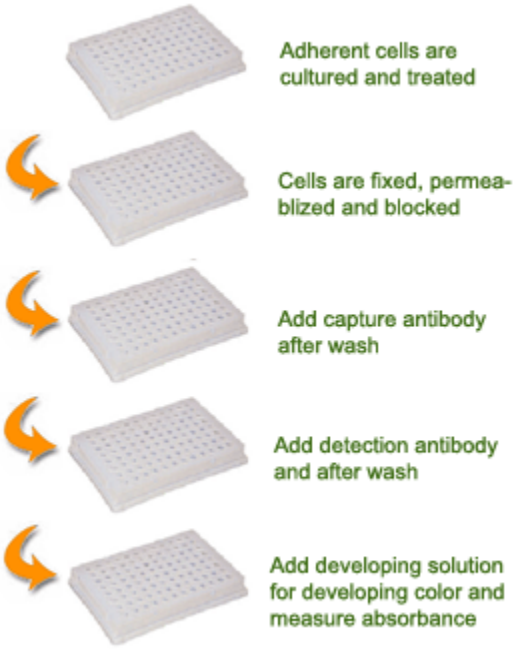
A BRIEF OVERVIEW

Acetylation of histones, including histone H3, have been involved in the regulation of chromatin structure and the recruitment of transcription factors to gene promoters. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) play a critical role in controlling histone H3 acetylation. Histone acetylation is tightly involved in cell cycle regulation, cell proliferation, and apoptosis. An imbalance in the equilibrium of histone acetylation has been associated with tumorigenesis and cancer progression. Histone H3 at lysine 9(H3-K9) acetylation is a mark of active chromatin and appears to have a dominant role in histone deposition and chromatin assembly in some organisms. Acetylation of H3-K9 may be increased by inhibition of HDACs and decreased by HAT inhibition. Increased acetylation of H3-K9 may also reflect a decrease in H3-K9 methylation associated with gene repression. The *EpiQuik™ In Situ* Histone H3-K9 Acetylation Assay Kit provides a convenient procedure to measure *in situ* acetylation of histone H3-K9. The kit has the following features:

- Quick and efficient procedure, which can be finished within 3 hours.
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography.
- Measurement of *in situ* histone H3-K9 acetylation without the need to prepare cell lysates.
- Microplate format makes the assay suitable for high throughput analysis of agents that increases or inhibit H3-K9 acetylation.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik™ In Situ* Histone H3-K9 Acetylation Assay Kit uses whole cell-based detection of acetylated H3-K9. In this assay, adherent cells are cultured in conventional 96-well microplates. After your experimental treatment, cells are fixed and permeabilized. The acetylated H3-K9 is then detected by a high affinity acetyl H3-K9 antibody. The ratio or amount of acetyl H3-K9 can be quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.



Schematic Procedure for Using the *EpiQuik™ In Situ* Histone H3-K9 Acetylation Assay Kit

PROTOCOL

Before starting, perform the following:

(A) Prepare the following required solution (not included): 37% Formaldehyde.
(B) Ensure that all buffer solutions are clear in appearance. Shake or vortex if these buffers contain precipitates.

1. Grow adherent cells in 96-well microplate to 50-60% confluency. Treat cells with the appropriate amount of reagents that may increase or reduce H3-K9 acetylation for the appropriate time, based on your experiment design.
2. Prepare the **Fixing Solution** by adding 2.16 ml of *37% Formaldehyde* to 18 ml of PBS. Remove culture media from the wells with a wrist-flick.
3. Immediately add 150 µl of the **Fixing Solution** slowly to the wells and incubate at room temperature for 15 minutes. Remove the **Fixing Solution** from the wells with a wrist-flick; while still inverted, tap the plate gently onto absorbent paper to remove any excess fixing reagent still within the wells.
4. Dilute **GH1** with distilled water (pH 7.2-7.5) at a 1:10 ratio (e.g., 1 ml of **GH1** + 9 ml of distilled water). Wash the wells once (for 2 minutes) with 150 µl of **diluted GH1**.
5. Remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Add 150 µl of **GH2** to each well and incubate at room temperature for 5 minutes. Meanwhile, prepare the **1% H_2O_2 Solution** by adding 330 µl of **30% H_2O_2** into 10 ml of **GH2**.

6. Remove **GH2** from wells with a wrist flick and add 100 µl of the **1% H_2O_2 Solution** into each well and incubate at room temperature for 10 minutes to remove endogenous peroxidase.
7. Remove the **1% H_2O_2 Solution** from the wells with a wrist flick and wash the wells twice with 150 µl of the **diluted GH1** solution.
8. Remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Add 150 µl of **GH3** to the wells and incubate at 37°C for 45 minutes. Meanwhile, add 50 µl of the **diluted GH1** solution to the desired number of control strip wells, followed by adding 1 µl of **Acetylated H3-K9 Control** at the different amounts (ex: 0.5-20 ng, diluted with distilled water) and incubate at room temperature for 30-45 minutes. For the blank wells, do not add Acetylated H3-K9 Control.
9. Remove **GH3** with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Wash the wells twice with 150 µl of the **diluted GH1** solution.
   For each wash, remove the **diluted GH1** solution with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Meanwhile, aspirate the solution from the control strip wells and wash the wells with 150 µl of **diluted GH1** three times.
10. Dilute **GH5** (at a 1:1000 ratio) to 1 µg/ml with **GH4**. Add 50 µl of the **diluted GH5** to the sample wells and the **Acetylated H3-K9 Control Strip Wells**. Incubate at room temperature for 60 min on an orbital shaker (50-100 rpm).
11. Remove the solution from the wells with a wrist flick and wash the wells four times with 150 µl of **diluted GH1**. For each wash, remove the **Diluted GH1** with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution.
12. Dilute **GH6** (at a 1:1000 ratio) to 0.4 µg/ml with **GH4**. Add 50 µl of **diluted GH6** to the wells and incubate at room temperature for 30 minutes.
13. Remove the solution from the wells with a wrist flick and wash the wells four times with 150 µl of **diluted GH1**. For each wash, remove the **diluted GH1** with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution.
14. Add 100 µl of **GH7** to the wells and incubate at room temperature for 1-10 minutes away from light. Monitor the color development in the sample and control wells until you observe a medium blue color.
15. Add 50 µl of **GH8** to each well to stop enzyme reaction when the color in the standard wells containing the higher concentrations of standard control turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm within 2-15 minutes.
16. Calculate % H3-K9 Acetylation:

$$\text{Acetylation \%} = \frac{\text{O.D. (treated sample – blank)}}{\text{O.D. (untreated control – blank)}} \times 100\%$$

TROUBLESHOOTING

No Signal for Both the Positive Control and the Samples

Reagents are added incorrectly.	Check if reagents are added in the proper order and if any steps of the procedure may have been omitted by mistake.
Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.

No Signal for Only the Sample

Cells are not fixed and permeabilized sufficiently.	Ensure fixation solution and permeabilizing solution are sufficiently added into the wells and incubation time is enough.

High Background Present for the Blank

The well is not washed enough.	Check if wash at each step is performed according to the protocol.
Overdevelopment.	Decrease development time in Step 14.

RELATED PRODUCTS

P-4002	*EpiQuik*™ HDAC Activity/Inhibition Assay Kit (Colorimetric)
P-4003	*EpiQuik*™ HAT Activity/Inhibition Assay Kit (Colorimetric)
P-4005	*EpiQuik*™ HDAC1 Assay Kit (Colorimetric)
P-4006	*EpiQuik*™ HDAC2 Assay Kit (Colorimetric)
P-4007	*EpiQuik*™ HDAC8 Assay Kit (Colorimetric)
P-4008	*EpiQuik*™ Global Histone H3 Acetylation Assay Kit
P-4009	*EpiQuik*™ Global Histone H4 Acetylation Assay Kit

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